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UNITED STATES PATENT APPLICATION

For

SHAPED ILLUMINATION GEOMETRY AND INTENSITY USING A DIFFRACTIVE
OPTICAL ELEMENT

by

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I. FIELD OF THE INVENTION

[001] This invention relates to methods and optical systems for illuminating a target. The present invention also relates to methods and systems for performing sample assays, and for producing and measuring optical responses and signatures.

II. BACKGROUND OF THE INVENTION

[002] Targets, such as areas where optically transduced chemical and/or biochemical assays are performed, may need to be illuminated by a light source. It is often desirable to illuminate the target with light having enhanced uniformity of intensity over the entire target region. Optical signals are typically a function of the illumination intensity and, the more an illumination intensity varies across a target, the more the optical signal will also vary. The resultant variance in optical signals may be undesirable.

[003] However, it can be difficult to efficiently provide illumination having enhanced or a high degree of uniformity. For example, lasers, which are commonly used for illuminating targets, typically have an intensity profile that is peaked at its center and which drops off radially towards the edges. This intensity profile is often a Gaussian, or bell shaped, profile. Therefore, if a target is directly illuminated with such a laser, the illumination of the target will not have a constant intensity. Rather, the center portion of the target will receive greater illumination intensity than the perimeter areas.

[004] Therefore, there is a need for an apparatus and method to illuminate a target or selected area with enhanced uniformity as compared to directly illuminating the target with a given light source. Further, there is a need for an apparatus and

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method that provides enhanced illumination uniformity for optical targets such as those in chemical and/or biochemical assay systems.

III. SUMMARY OF THE INVENTION

[005] According to certain embodiments of the invention, an apparatus is provided to illuminate a target. The apparatus comprises a light source, a first lens, a diffractive optical element, and a second lens. The first lens is configured to receive light from the light source. The diffractive optical element is configured to receive the light from the first lens and to regulate the light into regulated light. The second lens is configured to receive the regulated light and to direct the regulated light onto a selected area of the target.

[006] According to certain embodiments of the invention, a method is provided to illuminate a target. The method comprises generating light from a light source, directing the light with a first lens to a diffractive optical element, generating regulated light with the diffractive optical element, and focusing the regulated light with a second lens onto a selected area of the target.

[007] According to yet another aspect of the present invention, the inventive apparatus and method provide non-normal angle of incidence illumination of a selected area with a given light source with a greater degree of uniformity than is achieved when that light source is used to directly illuminate the selected area at the same non-normal angle of incidence.

[008] According to certain embodiments of the present invention, the inventive apparatus and method are directed towards the analysis of a sample in which light is generated from a light source, the light is directed with a first lens to a diffractive

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optical element; regulated light is generated with the diffractive optical element; the regulated light is delivered onto a selected area of a target that comprises at least one optically active species; and changes in an optical signature of the at least one optically active species are detected.

[009] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the invention, as claimed.

IV. BRIEF DESCRIPTION OF THE DRAWINGS

[010] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the invention.

[011] Figure 1 is a schematic illustration of one embodiment of an apparatus of the present invention.

[012] Figure 2 is a schematic illustration of the illumination of a selected area at a non-normal angle of incidence, where the regulated light has a gradient intensity profile in order to provide substantially uniform illumination of the selected area at a non-normal angle of incidence.

[013] Figure 3 A and B illustrate the distortive effect of non-normal angle illumination of a selected area and the use of regulated light to compensate for this effect in order to more uniformly illuminate a selected area of a given shape.

[014] Figure 4 is a schematic illustration of an embodiment according to the present invention, having an optical diffuser for removing speckle arranged between the first lens and the diffractive optical element.

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V. DESCRIPTION OF CERTAIN EMBODIMENTS OF THE INVENTION

[015] Reference will now be made in detail to certain embodiments of the invention, examples of which are illustrated in the accompanying drawings. Wherever possible, the same reference numbers will be used throughout the drawings to refer to the same or like parts.

[016] The section headings used herein are for organizational purposes only, and are not to be construed as limiting the subject matter described. All documents cited in this application, including, but not limited to patents, patent applications, articles, books, and treatises, are expressly incorporated by reference in their entirety for any purpose.

[017] It should be understood that the phrases "uniform illumination" and "uniformly illuminate," as used herein with respect to the illumination of a selected region, refer to the variation in optical intensity of the light across the selected region. The lower the variation, the more uniform the illumination. Thus, uniform illumination can be characterized qualitatively as a relative measurement. The variation of the illumination can also be characterized quantitatively, for example, by the relative deviation of the optical intensity ("intensity variation") across the selected region, with smaller intensity variations meaning that the illumination has a higher degree of uniformity.

[018] The relative deviation of the intensity can be found from the ratio of the standard deviation of the intensity to the mean value of the intensity, each measured within the selected region. The relative deviation may also characterize a scaled intensity variation, which is calculated from the ratio of the standard deviation of a scaled intensity to the mean value of the scaled intensity, each measured within the

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configured to receive light from the light source. The diffractive optical element is configured to receive the light from the first lens and to regulate the light into regulated light. The second lens is configured to receive the regulated light and to direct the regulated light onto a selected area of the target.

[034] According to certain embodiments, the present invention provides a method to illuminate a target. This method comprises generating light from a light source, directing the light with a first lens to a diffractive optical element, generating regulated light with the diffractive optical element, and focusing the regulated light with a second lens onto a selected area of the target.

[035] Figure 1 schematically illustrates an apparatus according to certain embodiments of the invention. Light source 10 emits light 20, which is received by the first lens 30. The light 20 is directed by first lens 30 to the diffractive optical element 40. In these illustrative embodiments, the light 20 emitted from the light source 10 is divergent with an angle of divergence α , and has an intensity profile that is brightest in the center, as shown by the shading profile, which is darkest at the center. In these illustrative embodiments, the first lens 30 is also configured to collimate the light 20, as shown schematically.

[036] The diffractive optical element 40 receives the light 20 and regulates it into regulated light 50. In this illustrative embodiment, the regulated light 50 has an intensity profile that is substantially uniform, as shown by the substantially uniform shading profile. In these embodiments, the diffractive optical element 40 is also configured to reshape the light 20 from an elliptical shape into rectangular shaped regulated light 50. The second lens 60 receives the regulated light 50 and directs it

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Additional considerations for each of these elements will also be discussed below.

[039] According to certain embodiments, the light source may be wholly or partially coherent or incoherent, and may be chosen from, for example, at least one of the following non-limiting examples: a laser, an electroluminescent light source, a chemoluminescent light source, an electrochemoluminescent light source, an incandescent light source, a fluorescent light source, an arc lamp, and a light emitting diode. According to certain embodiments, the light source may also be chosen from continuous wave (CW) and pulsed lasers and from gas, solid state, fiber optical, and organic based lasers.

more light sources. For example, the light source may comprise a first light source

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(IFTAs). IFTAs can be used to design diffractive optical elements producing any desired intensity distribution in the diffraction plane based on any intensity cross-section of the incident beam. See, for example, M. Johansson et al., "Robust design method for highly efficient beam-shaping diffractive optical elements using an iterative-Fourier-transform algorithm with soft operations," *Journal of Modern Optics*, 47(8), 1385 - 1398 (2000), the entirety of which is incorporated herein by reference for any purpose. According to certain embodiments, optical elements, and their combinations, including, for example, diffractive optical elements, can be designed using software packages, such as, for example, ZEMAX® from Focus Software, Inc.

[056] Diffractive optical elements may include, e.g., holograms and holographic optical elements. According to certain embodiments, suitable diffractive optical elements include, for example, those chosen from amplitude (e.g., absorption) and phase holograms; optically etched diffractive optical elements; embossed diffractive optical elements; molded diffractive optical elements; chemically etched diffractive optical elements; thin or surface (2-dimensional) holographic optical elements and volume (3-dimensional) holographic optical elements; reflection and transmission holograms; multiplex holograms; rotating holograms, such as, for example, a rotating disc composed of a series of holographic optical elements that diffracts light at various angles, when spinning, for example, to generate a raster scan; Fresnel holograms; and combinations thereof.

[057] According to certain embodiments, the diffractive optical element is configured to regulate the received light and compensate for at least one of light intensity distributions and shapes of the light due to at least one of the light source

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configured to regulate received light having shapes other than circular, and to generate regulated light having shapes other than rectangular.

[060] For example, as shown in Figure 1, light 20 having an elliptical cross-section and an intensity distribution peaked in the center (as shown by darkened central shading) is regulated by the diffractive optical element 40 and regulated into regulated light 50 that has a rectangular cross-section and a constant intensity distribution (as shown by uniform shading). Of course, as discussed above, according to certain embodiments the light need not be regulated into regulated light having a constant intensity distribution.

[061] The resultant intensity re-distributed and/or re-shaped light may or may not be immediately suitable for the illumination of the selected area of the target. For example, in certain embodiments, the diffractive optical element may be configured to not only post-compensate for effects of elements optically prior to the diffractive optical element but also to pre-compensate for effects of elements optically subsequent to the diffractive optical element. Thus, according to certain embodiments, the regulated light is most suitable for illumination of the selected area after it interacts with the second lens and/or other subsequent elements.

Additionally, according to certain embodiments, the regulated light may be designed to allow for its propagation, including any changes in size, shape, and intensity distributions that result therefrom, prior to its illumination of the selected area.

[062] According to certain embodiments, the diffractive optical element may be configured to produce regulated light that is less uniform than the received light but which, after interacting with subsequent optical elements and/or further propagation,

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embodiments, the regulated light may be designed to compensate for the illumination of the selected area at a non-normal angle of incidence.

[067] To achieve substantially uniform illumination of a target, such as a tilted target, in certain embodiments, the diffractive optical element is configured to provide regulated light that has an intensity gradient. For example, according to certain embodiments, as shown by shading in Figure 2, when the regulated light is converging towards the target at a non-normal angle of incidence, the intensity profile of the regulated light 50 is greater towards one edge (as shown by the darker shading, which indicates greater light intensity) in order to provide substantially uniform illumination intensity across the selected area 75 of the target 70, which is illuminated at an angle tilted from normal by angle β . In certain embodiments, the intensity gradient may be proportional to the tilt angle, with the graded intensity increasing towards the edge furthest away from the selected area.

[068] According to certain embodiments, the apparatus may be configured to illuminate a selected area of a given shape at a normal or non-normal angle of incidence. For example, in certain embodiments, there can be non-normal illumination. In certain embodiments, if the selected area is, for example, square shaped, the regulated light would be shaped such that, when incident on the selected area at the non-normal angle of incidence, the light illuminates a square shaped area. Due to the non-normal angle of incidence, however, the regulated light will not necessarily have a square shaped profile.

[069] According to certain embodiments, the non-normal angle illumination of a selected area with light of a given cross sectional profile has analogies with the

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illuminate the selected area with an intensity variation of less than 25%. In certain embodiments, the present invention may be configured to direct any percent between 10% and 100% percent of the light from the light source onto the selected area and to illuminate the selected area with an intensity variation of any percent less than 25%.

[074] In certain embodiments of the present invention, the apparatus may be configured to control a numerical aperture of the regulated light directed onto the selected area. In certain embodiments, such a design can be used to produce a selected depth of field and a selected edge sharpness.

[075] According to various embodiments, virtually any target or selected area may be illuminated. For example, according to certain embodiments, the target may comprise at least one optical fiber bundle, including an optical fiber bundle comprising separate wells at terminal ends of optical fibers of the bundle, and including optical fiber bundles such as those disclosed in U.S. Patent No. 6,023,540 to Walt et al., which is incorporated by reference herein in its entirety for any purpose. According to certain embodiments, the target may comprise at least one microcard, including, for example, those sold under the trade name TAQMAN® CYTOKINE GENE EXPRESSION CARDS by Applied Biosystems. According to certain embodiments, the target may comprise at least one of a substrate, fluidics network, and device, such as those disclosed in U.S. Patent No. 6,126,899; WO Application No. 97/36681; and U.S. Patent No. 6,272,939, the disclosures of which are incorporated herein by reference in their entirety for any purpose. According to

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[079] According to certain embodiments, the selected area of the target may have any shape, and may or may not be a single continuous area. For example, the selected area may be rectangular, including approximately 1mm x 1.5 mm.

[080] According to certain embodiments, the selected area may comprise at least two or more spatially separate areas. For example, the selected area may be at least two wells separated by some distance, and, in certain embodiments of the present invention, the spatially separate wells but not the area between the wells, will be illuminated. In certain embodiments, the selected area may comprise the wells of a microtiter plate chosen from microtiter plates having 96, 128, 384, and 1536 wells. In certain embodiments, for example, the selected area may comprise multiple well plates such as those sold by Applied Biosystems under the trade names TAQMAN® CYTOKINE GENE EXPRESSION CARDS MICROAMP® 384-WELL REACTION PLATES, and MICROAMP® 96-WELL TUBES/TRAY/RETAINER ASSEMBLIES. According to certain embodiments, the spatially separate areas may be illuminated simultaneously, sequentially, and/or any combination thereof. In certain embodiments, rows of spatially separate areas may be illuminated sequentially.

[081] According to certain embodiments, an apparatus is provided that can illuminate a target having microwells. In certain embodiments, the apparatus comprises a target comprising microwells, a light source, a first lens configured to receive light from the light source, a diffractive optical element configured to receive the light from the first lens and to regulate the light into regulated light, and a second lens configured to receive the regulated light and to direct the regulated light onto a

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[084] According to certain embodiments, the probes include fluorescent molecules attached to fluorescence quenching molecules by a short oligonucleotide. In certain embodiments, the probes with the fluorescent molecules bind to the target molecule, but are broken by the 5' nuclease activity of the DNA polymerase when they are replaced by the newly polymerized strand during PCR, or some other strand displacement protocol. When the oligonucleotide portion is broken, the fluorescent molecule is no longer quenched by the quenching molecule, and emits a fluorescent signal. An example of such a system has been described in U.S. Patent No. 5,538,848, which is incorporated herein by reference, and is exemplified by the TaqMan™ molecule, which is part of the TaqMan™ assay system (available from Applied Biosystems).

[085] According to certain embodiments, the probes may be "molecular beacons," which comprise a fluorescent molecule attached to a fluorescence-quenching molecule by an oligonucleotide. When bound to a polynucleotide as double stranded nucleic acid, the quenching molecule is spaced apart from the fluorescent molecule, and the fluorescent indicator may give a fluorescent signal. When the molecular beacon is single stranded, the oligonucleotide portion can bend flexibly, and the fluorescence-quenching molecule can quench the fluorescent molecule, reducing the amount of fluorescent signal. Such systems are described in U.S. Patent No. 5,723,591, which is incorporated herein by reference.

[086] According to certain embodiments, an apparatus is provided that can illuminate a target configured for at least one of hybridization and electrophoresis. Hybridization is the pairing of complementary nucleic acid strands to make double

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selected area of the target. A target array is understood to be a target composed of more than one sub targets. Non-limiting examples of target arrays include the multiple fiber wells disclosed in *Walt et al.*; U.S. Patent No. US 6,023,540; J.A. Ferguson *et al.*, *Analytical Chemistry*, 72, 5618 (2000); F.J. Steemers *et al.*, *Nature Biotechnology*, 18, 91-94 (2000); and D.R. Walt, *Science*, 287, 451-452 (2000), the disclosures of which are incorporated herein by reference in their entirety for any purpose.

[088] According to certain embodiments an apparatus is provided that is configured to perform an assay on a sample. In certain embodiments, the apparatus comprises a target configured to receive the sample, a light source, a first lens configured to receive light from the light source, a diffractive optical element configured to receive the light from the first lens, and to regulate the light into regulated light, and a second lens configured to receive the regulated light and to direct the regulated light onto a selected area of the target. According to various embodiments, the sample may be in any form, such as solid, liquid, gas, and mixtures thereof. Non-limiting examples of such samples include blood and samples derived from blood, samples of proteins, samples of nucleic acids, air samples, and/or solutions comprising antibodies and/or antigens. According to certain embodiments, at least one of the target and the sample comprises at least one optically active species. According to certain embodiments, at least one of the target and the sample comprises at least one fluorescent species.

[089] According to certain embodiments, the sample may comprises a "biological sample," which is used in a broad sense and is intended to include a variety of

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biological sources that contain nucleic acids. Such sources include, without limitation, whole tissues, including biopsy materials and aspirates; *in vitro* cultured cells, including primary and secondary cells, transformed cell lines, and tissue and cellular explants; whole blood, red blood cells, white blood cells, and lymph; and body fluids such as urine, sputum, semen, secretions, eye washes and aspirates, lung washes and aspirates. According to certain embodiments, samples comprising fungal and/or plant tissues, such as leaves, roots, stems, and caps, are also within the scope of the present invention. According to certain embodiments, samples comprising microorganisms and/or viruses that may be present on or in a biological sample are within the scope of the invention.

[090] According to certain embodiments, an apparatus is provided that is configured to perform an assay on a sample. According to certain embodiments, as shown in Figure 4, the apparatus comprises a target 70 configured to receive the sample. According to certain embodiments, the target may comprise a sample holder and/or a sample. The apparatus further comprises a light source 10, a first lens 30 configured to receive light 20 from the light source 10, and a diffractive optical element 40. The diffractive optical element 40 is configured to receive the light 20 from the first lens 10, to regulate the light 20 into regulated light 50, and to direct the regulated light onto the selected area 75 of the target 70. As illustrated by the gradated shading of regulated light 50, the regulated light may have an intensity gradient to provide more uniform illumination intensity (as compared with light having no intensity gradient) to the selected area 75 when illuminated at a non-normal angle of incidence, β . In certain embodiments, the regulated light 50 may be shaped with

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a shape that matches the selected area after illumination of the selected area at a non-normal angle of incidence. According to various embodiments, a second lens, configured to direct the regulated light onto the selected area, is optional.

[091] According to certain embodiments, as shown in Figure 4, the selected area 75 may be a well, such a well configured to receive a sample. According to certain embodiments, at least one of the target and the sample comprises at least one optically active species 90. According to certain embodiments, the optically active species 90 comprises at least one fluorescent species.

[092] In certain embodiments, an apparatus optionally may include an optical diffuser configured to remove speckle, such as speckle due to the interference of coherent light. According to certain embodiments, as shown in Figure 4, an optical diffuser 80 may be located between the first lens 30 and the diffractive optical element 40. The optical diffuser, however, may be placed anywhere within the apparatus. According to certain embodiments, the optical diffuser may be any suitable optical element which is useful for removing speckle. In certain embodiments, the optical diffuser may comprise at least one of a rotating optical diffuser and a light shaping optical diffuser (LSD), such as, for example, an LSD comprising surface relief holograms with random, non-periodic structures. In certain embodiments, one may use optical diffusers from Physical Optical Corporation, including those with the catalog numbers LSD-KIT-CN-x-y, where x is a diffuser angle chosen from 0.5, 1, 5, and 10° and where y is a diameter chosen from 25 and 50 mm.

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[096] It is intended that the specification and examples be considered as exemplary only, with a true scope and spirit of the invention being indicated by the following claims.

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